ASPECTS OF OSMOREGULATION IN TWO SPECIES OF INTERTIDAL CRABS[1]

PAUL A. DEHNEL

Department of Zoology, University of British Columbia, Vancouver 8, British Columbia

Considerable attention has been given problems of osmotic behavior in decapod Crustacea. Much of this work has concerned intertidal and semi-terrestrial species. Less information is available for species inhabiting estuarine conditions. Krogh (1939) and Prosser and Brown (1961) have reviewed extensively the subject of osmotic and ionic regulation. In the last several years Gross (1955, 1957a, 1957b, 1958, 1959) has contributed significantly to the understanding of water and ion balance in several species of crabs, with particular emphasis on *Pachygrapsus crassipes.*

For the past few years efforts have been made to understand aspects of the physiology of *Hemigrapsus oregonensis* and *H. nudus* (Dehnel, 1960). This previous work has shown that oxygen consumption measurements for animals maintained at a constant acclimation temperature, but a series of acclimation salinities, are higher at the lower salinities. This work has led to the suggestion that this increased respiratory rate at low salinities is the result of increased osmotic work, and not the result of increased muscular activity. Increased oxygen consumption has been demonstrated for crabs kept in an osmotic stress (Schlieper, 1929; Schwabe, 1933; Flemister and Flemister, 1951). These workers have interpreted their data to mean that increased oxygen consumption reflects increased osmotic work. Krogh (1939) and Wikgren (1953) tend to refute this idea. Gross (1957a) presents evidence also to the contrary, interpreting the results in terms of increased muscular activity.

It seems reasonable that both ideas are valid, depending upon the animal in question. *Pachygrapsus crassipes,* for instance, probably never encounters hypotonic environmental conditions. Both species of *Hemigrapsus* in this geographic region are exposed always to low salinity conditions during the summer months, in conjunction with relatively high temperatures. The reverse is true during the winter, although the sea water salinity is lower than 100%. Because of seasonal variation in salinity it would appear quite probable that *Hemigrapsus* would be a strong hyper-osmotic regulator, and as the osmotic gradient increased between blood and sea water, additional work would be necessary in order to maintain the gradient. This might be reflected in the oxygen consumption of the whole animal. The problem is to detect increased metabolic work, by measuring oxygen consumption, and to relate it to increased osmotic work. As yet, this relationship has not been resolved.

[1] These studies were aided by grants from the National Research Council of Canada and the National Science Foundation of the United States. The author wishes to express his gratitude to Dr. Warren J. Gross for his critical reading of the manuscript.

The present study has determined the osmotic behavior of the two species of *Hemigrapsus* when exposed to a series of salinities, as well as to changing seasonal field salinities. Further, the effect of temperature on osmotic regulation was investigated, by exposing crabs to various combinations of temperature and salinity.

Material and Methods

Both species of crabs used for this study were collected from Spanish Bank, Vancouver, British Columbia. Experiments were conducted at a series of temperatures and salinities on animals collected from summer and winter populations. Seasonal field variation in these two environmental parameters has been described (Dehnel, 1960). Winter animals were returned to the laboratory and placed directly into an experimental temperature, but at a holding salinity of 75% sea water for 18 hours maximum. Crabs were transferred then to the experimental salinity and temperature conditions. Summer animals followed the same sequence except that these animals were maintained at the holding salinity for approximately 30 hours. Winter and summer animals were placed initially in 75% sea water to permit a common salinity facilitating comparison for all experimental series. Animals were placed directly into the experimental temperatures except for 5° and 25° C.; these were placed in room temperature water (16° to 18° C.) for approximately three hours, then into the controlled-temperature refrigerators. Throughout the series of experiments crabs were kept in the dark and not fed. Crabs were maintained in plastic containers, approximately 30 animals per container. Water of the appropriate salinity and temperature was changed daily. Crabs were fully immersed during the experimental periods.

Experimental temperatures used were 5°, 10°, 15°, 20° and 25° C. (± 1.0° C.) and experimental salinities were 6, 12, 25, 75, 100, 125, 150 and 175% sea water. Salinities above 100% sea water were obtained by adding appropriate amounts of reagent sodium chloride. Salinities below 100% were obtained by adding distilled water to normal sea water. All field and experimental salinities are expressed as percentage sea water, based on a standard sea water, 31.88‰ salinity, 17.65‰ chlorinity at 25° C. as 100% sea water. Salinities were determined on a 1000-cycle conductivity bridge calibrated to the standard sea water noted above.

Melting points of sea water concentrated by several methods were determined to establish whether total osmotic pressure differed. Boiling and freezing low salinity water to 100% sea water resulted in essentially the same value. Sea water concentrated to 150% by addition of NaCl, then diluted to 100% with distilled water gave a somewhat higher but statistically insignificant value when compared with the other two methods. Similarity of melting point values would suggest that the ion balance of concentrated sea water was not significantly altered by addition solely of NaCl.

Blood was sampled from the crabs by two methods: removal of the coxopodite of the last pereiopod, or puncture of the membrane proximal to the coxopodite. The area was damp-dried and blood was allowed to flow from the region of removal or puncture. This area was dried again and then the blood was sampled. The sample was collected in a blood capillary tube (0.4 mm. I.D.), sealed with Nevastane grease and frozen on dry ice. Puncture of the membrane permitted

the animal to be returned to the experimental conditions for further sampling. Animals from which a leg was removed were discarded. The two sampling techniques produced identical results, as the puncture closed rapidly. The puncture technique was used on summer animals to facilitate judicious use of the crab population. At the time of each collection, blood from a group of animals was sampled immediately upon return to the laboratory. The blood concentrations of these animals provided data which demonstrated periodic blood changes, due to variations of field salinity. Field temperature and salinity data were obtained for each collection. Blood of crabs held at the experimental conditions was sampled at three, 24 and 48 hours following placement into the experimental conditions. A modified method for melting point determination, as described by Gross (1954), was used to determine total osmotic pressure. Ten to 15 animals were sampled to determine osmotic pressure of the blood at each time period. Points on the various graphs represent a mean of the measurements for each period.

For all experimental series a weight range was selected. Weight of summer animals ranged from approximately 1.0 gram to 5.0 grams; winter animals, from 0.5 gram to 5.0 grams. During the winter a parallel series of experiments was conducted to determine whether a change in total body weight could be detected in hypo- and hypertonic media. Crabs were damp-dried and weighed before and after exposure to the experimental conditions. Further, dry weights were determined for groups of crabs removed directly from field conditions, and for ones after exposure to the experimental conditions.

In Figures 1, 2, 5 and 6, per cent sea water of the body fluid of each species (summer and winter) at time zero was based on the mean of all three interval readings at 75% sea water for all temperatures. The difference between summer and winter groups of each species was small.

The term "gradient" is used to indicate the difference in concentration (expressed in per cent sea water) between the blood and the external medium. The data permitted use of the Student's "t" test for statistical treatment. Significance is considered at the 0.01 level of probability.

Results

Hemigrapsus oregonensis

Response to external salinity changes

The change in blood concentration as effected by different external sea water concentrations is shown in Figures 1 (summer) and 2 (winter). Results for summer animals were determined at 15° C. and those for the winter, at 5° C. These two temperatures approximate field temperature conditions for the two seasons. In general, changes in blood concentration were rapid; approximately one-half of the total change occurred within the first three hours. At the end of 24 hours, total blood concentration changes for summer animals (Fig. 1) had occurred in 100% to 6% sea water, and were hypertonic to these external media. After a 48-hour period, changes in the blood of animals in 125% and 150% sea water were not complete, but all were hypertonic to the external sea water. Crabs kept in 175% sea water died, following the three-hour period of exposure. The results for winter animals (Fig. 2) showed the blood to be hypertonic at all external

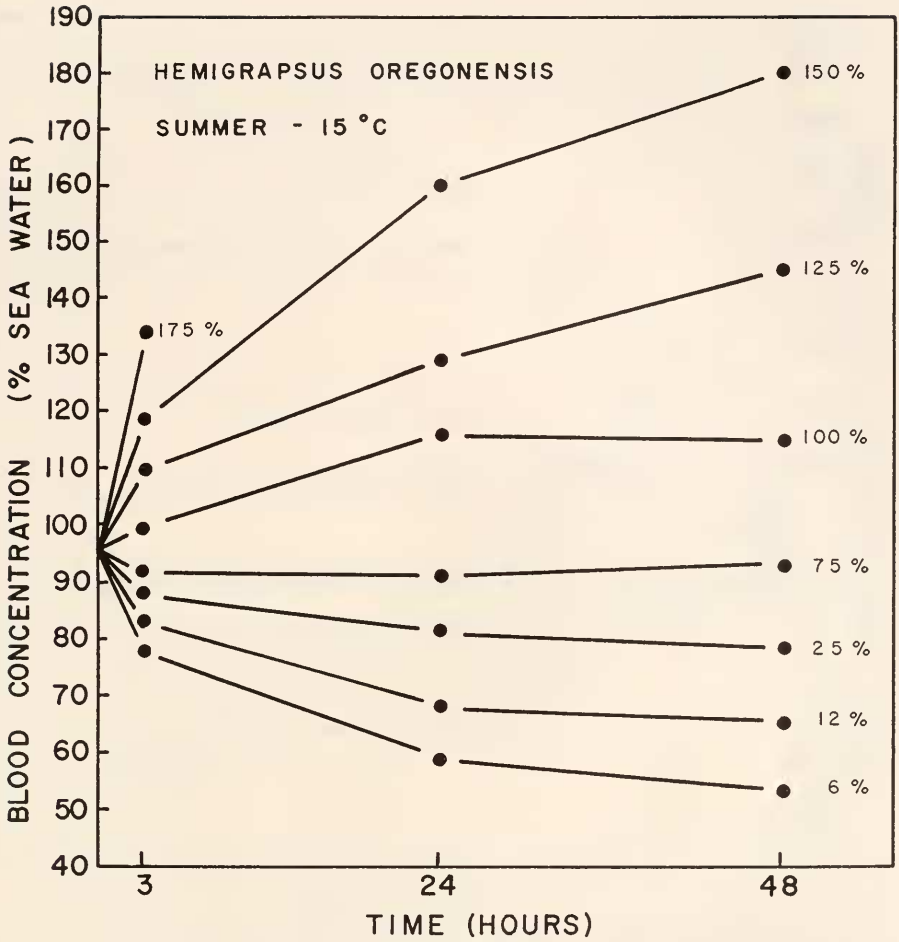

FIGURE 1. Osmotic regulation in summer *Hemigrapsus oregonensis,* at 15° C., as a function of time in the experimental salinities. Each point represents the mean of the measurements of 10 to 15 animals for each time period. Sea water concentrations are indicated in per cent normal sea water for each of the respective curves.

salinities, reaching a steady state after 24 hours' exposure, except 175% sea water. At this concentration the blood was still changing after the 48-hour period of exposure. It was assumed, however, that all major changes in blood concentration had occurred at the end of 48 hours.

Seasonal effect of temperature

The effect of temperature (5°, 15° and 25° C.) on the blood concentration for summer and winter animals exposed to different salinities is given in Figure 3. These results showed that blood concentrations were hypertonic to all external salinities at all temperatures. Data for 10° and 20° C. (not presented) are further documentation. At salinities less than 100% sea water, blood concentration of

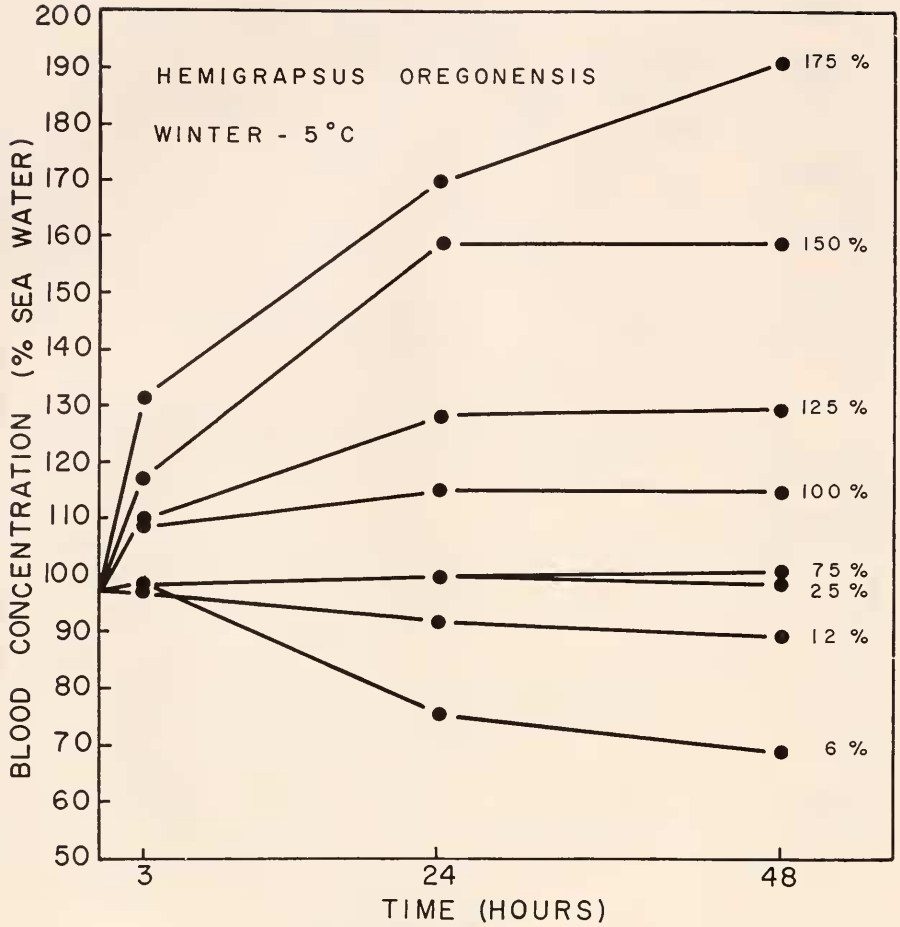

FIGURE 2. Osmotic regulation in winter *Hemigrapsus oregonensis*, at 5° C., as a function of time in the experimental salinities. Each point represents the mean of the measurements of 10 to 15 animals for each time period. Sea water concentrations are indicated in per cent normal sea water for each of the respective curves.

winter animals at the two lower temperatures was higher than that of summer animals at any of the temperatures ($P = 0.01$). At 100% sea water, blood concentrations for summer and winter animals for all temperatures were similar. Above 100% sea water the blood concentration for summer animals at 5° and 15° C. was significantly higher ($P = 0.01$), when compared with winter crabs.

Comparison of blood concentration of summer animals at all temperatures and at each salinity showed some difference (Fig. 3). For any salinity, the effect produced by the maximum temperature difference (20° C.) was of the order of 10%. When the summer 5° C. and 25° C. curves are compared at various salinities, the differences are statistically significant at 6, 100 and 125% sea water ($P = 0.01$). The same comparison for winter animals gave a somewhat greater

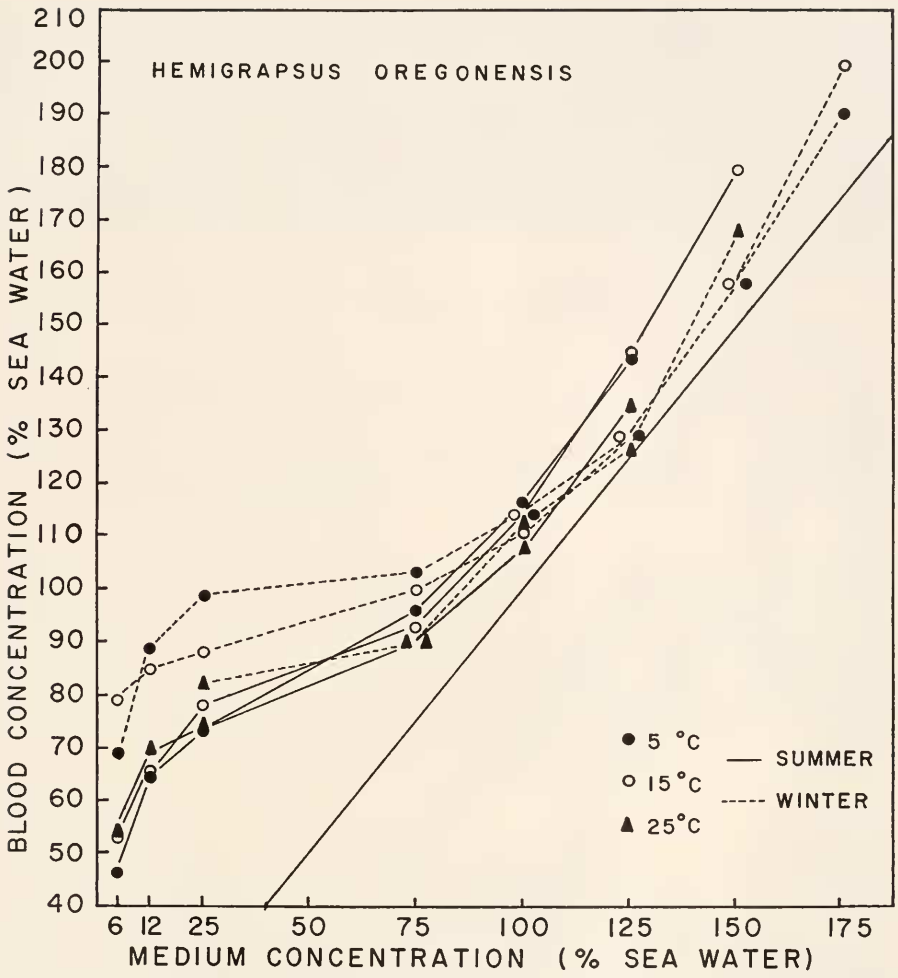

FIGURE 3. Relationship of the body fluid concentration of summer and winter *Hemigrapsus oregonensis*, at 5°, 15° and 25° C., to the medium concentrations after exposure for 48 hours to the experimental salinities.

concentration difference at salinities below 100% sea water. For example, at 25% sea water, the absolute difference between 5° and 25° C. was 17% ($P = 0.01$). As external salinity increased, the difference in blood concentration between low and high temperatures decreased, and a minimum was reached between 100% and 125% sea water for both summer and winter crabs. Above this concentration the difference increased slightly. It should be noted that at the lower salinities (12% to 75% sea water) the highest blood concentration, for winter animals in particular, was found generally at the lowest temperature (5° C.), *i.e.*, as the concentration of the external sea water decreased, blood concentration increased as temperature decreased.

When winter animals (Fig. 3) at their own approximate temperature and salinity field conditions (5° C.; 75% sea water) were compared with summer crabs at their corresponding conditions (15° C.; 25% sea water), blood concentration of winter crabs was more hypertonic (25% sea water difference; $P = 0.01$). When the blood of winter crabs was tested immediately upon removal from the intertidal zone, its concentration was always higher than that of summer crabs similarly removed. Further, comparison at either season showed the blood concentration always to be higher than the intertidal sea water concentration from which they were removed.

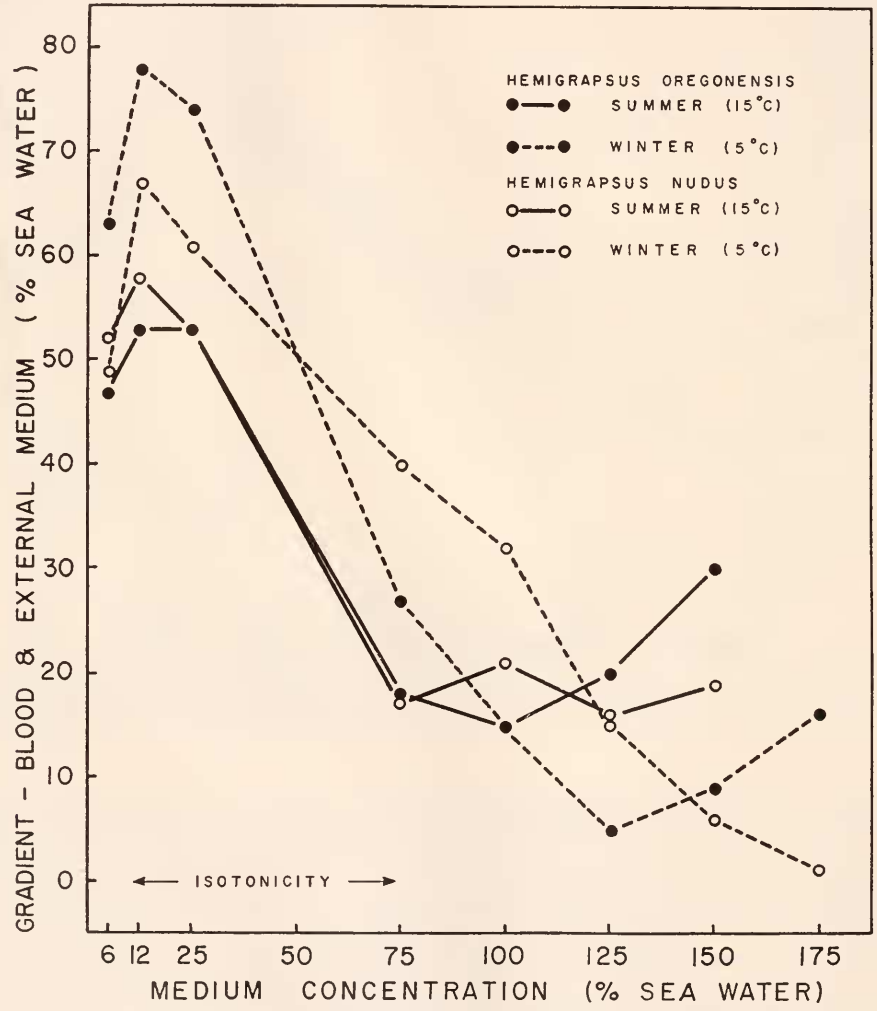

FIGURE 4. Relationship of the gradient between blood and external sea water to medium concentrations in summer (15° C.) and winter (5° C.) *Hemigrapsus oregonensis* and *Hemigrapsus nudus*, after exposure for 48 hours to the experimental salinities.

Figure 4 compares the concentration gradients between blood and sea water for summer and winter *H. oregonensis* at their approximate seasonal intertidal temperatures. These data showed that winter crabs maintained a greater gradient ($P = 0.01$) at all sea water concentrations below 100%. Above 100% sea water summer crabs maintained the greater gradient ($P = 0.01$). At 100% sea water summer crabs showed the greatest reduction in gradient, whereas winter animals approximated isotonicity at 125% sea water. Both groups increased steadily with increasing concentration of the external medium, but summer crabs had the greater absolute increase. The largest gradient between blood and external medium for summer and winter animals was at 12% sea water. For instance, winter *H. oregonensis* at 12% sea water maintained a 78% gradient, summer crabs, a 53% gradient, a statistically significant difference of 25%. Winter crabs are better regulators in hypotonic media and summer crabs are better regulators in hypertonic media. The differences at each salinity between summer (15° C.) and winter (5° C.) crabs are significant ($P = 0.01$) except at 100% sea water.

Data in Figure 3 show blood concentrations of summer and winter animals hypertonic to all external salinities. These blood concentrations, when compared with the respective external salinity, were significant, except for winter animals at 25° C. and 125% sea water.

Mortality occurred at some of the temperature and salinity combinations. Generally, at the higher salinities (150% and 175% sea water) summer animals died at the higher experimental temperatures (15° and 25° C.) during the 24-hour period of exposure. At 5° and 10° C. crabs survived the first day but died during the second day. Winter *H. oregonensis* were more resistant, and deaths occurred only at higher temperatures (20° and 25° C.) when exposed to the highest and lowest salinities, and only then after the 24-hour exposure period.

Hemigrapsus nudus

Response to external salinity changes

Blood concentration changes for summer (15° C.) and winter (5° C.) populations of this species, as effected by different external salinities, are presented in Figures 5 (summer) and 6 (winter). Major changes were rapid as in *H. oregonensis,* and occurred within three hours. After 24 hours, the maximum change for summer crabs essentially was reached (Fig. 5), and the blood of these crabs was hypertonic to all experimental salinities. Crabs exposed to 175% sea water died after three hours. Winter crabs showed less initial change and following 24 hours of exposure, changes still were evident (Fig. 6). Concentration of blood of winter crabs was hypertonic to all external media, but at 150% and 175% sea water blood approached isotonicity.

Seasonal effect of temperature

Seasonal temperature effect (5°, 15° and 25° C.) on the blood concentration of summer and winter *H. nudus* when exposed to different external salinities is shown in Figure 7. Blood concentrations were hypertonic at all external salinities and temperatures. However, at 150% and 175% sea water, blood of winter crabs at 5° and 25° C. was not significantly different from the line of isotonicity. Over the salinity range, 6% to 125% sea water, blood concentrations for summer or

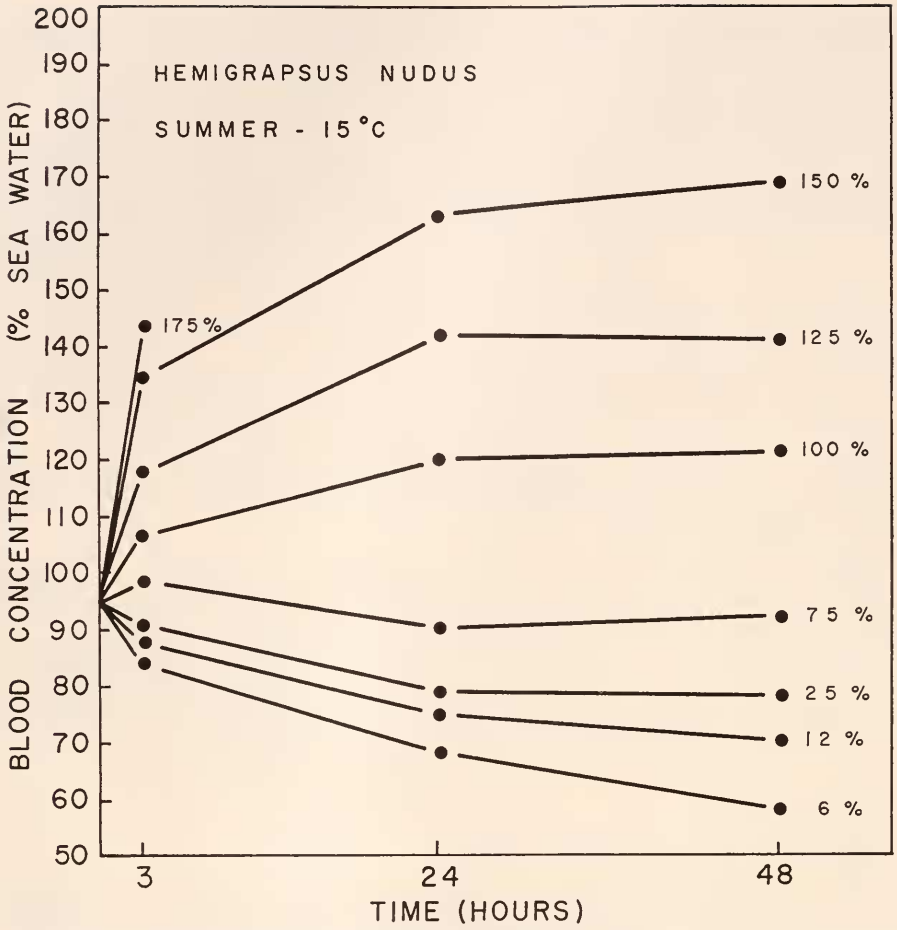

FIGURE 5. Osmotic regulation in summer *Hemigrapsus nudus* at 15° C., as a function of time in the experimental salinities. Each point represents the mean of the measurements of 10 to 15 animals for each time period. Sea water concentrations are indicated in per cent normal sea water for each of the respective curves.

winter crabs at the three temperatures were similar and not statistically significant, except for the 5° C. winter curve. This curve is noticeably higher throughout the intermediate range of salinities. At 125% sea water and above, the 5° C. curve loses its singular identity and the three temperature curves for winter animals are lower when compared with those of summer crabs. There was no real difference at each salinity when the blood concentration curves for summer crabs were compared at the three temperatures. The greatest difference in concentration effected by the maximum temperature difference (20° C.) was 14%, at a salinity of 12% sea water ($P = 0.01$), the usual being in the order of 5%. A greater blood concentration difference existed for winter animals over the range of salinity, 25% to 100% sea water. For instance, at 75% sea water, blood concentration differ-

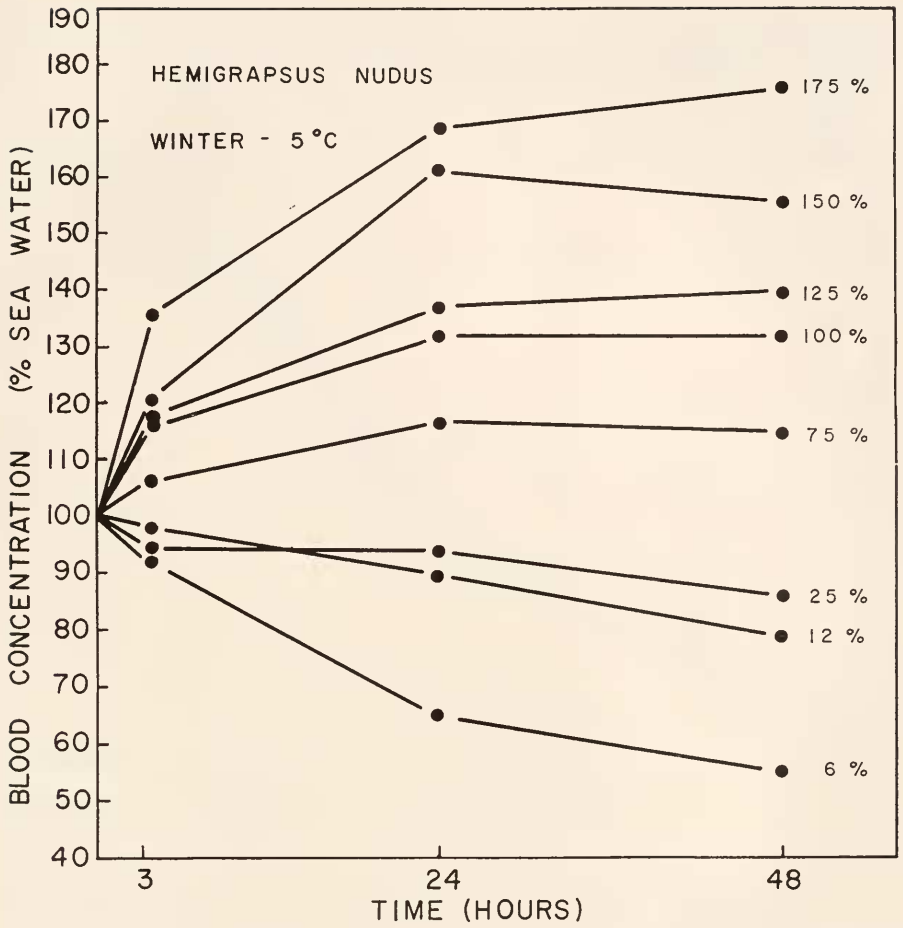

FIGURE 6. Osmotic regulation in winter *Hemigrapsus nudus*, at 5° C., as a function of time in the experimental salinities. Each point represents the mean of the measurements of 10 to 15 animals for each time period. Sea water concentrations are indicated in per cent normal sea water for each of the respective curves.

ence that resulted from the maximum temperature difference was 20% ($P = 0.01$). Minimum differences for winter animals ranged between 125% and 150% sea water. For summer animals this minimum difference occurred between 75% and 100% sea water. At the lower salinities, both summer and winter groups, the highest blood concentration generally was found at the lowest temperature, even though the temperature effect on blood concentration at a given salinity was relatively slight for summer animals.

Comparison of winter animals (Fig. 7) at their own temperature and salinity field conditions (5° C.; 75% sea water) with summer crabs (15° C.; 25% sea water) showed that winter crabs were more hypertonic (37% sea water difference; $P = 0.01$). Immediate testing of the blood of winter crabs, when removed from

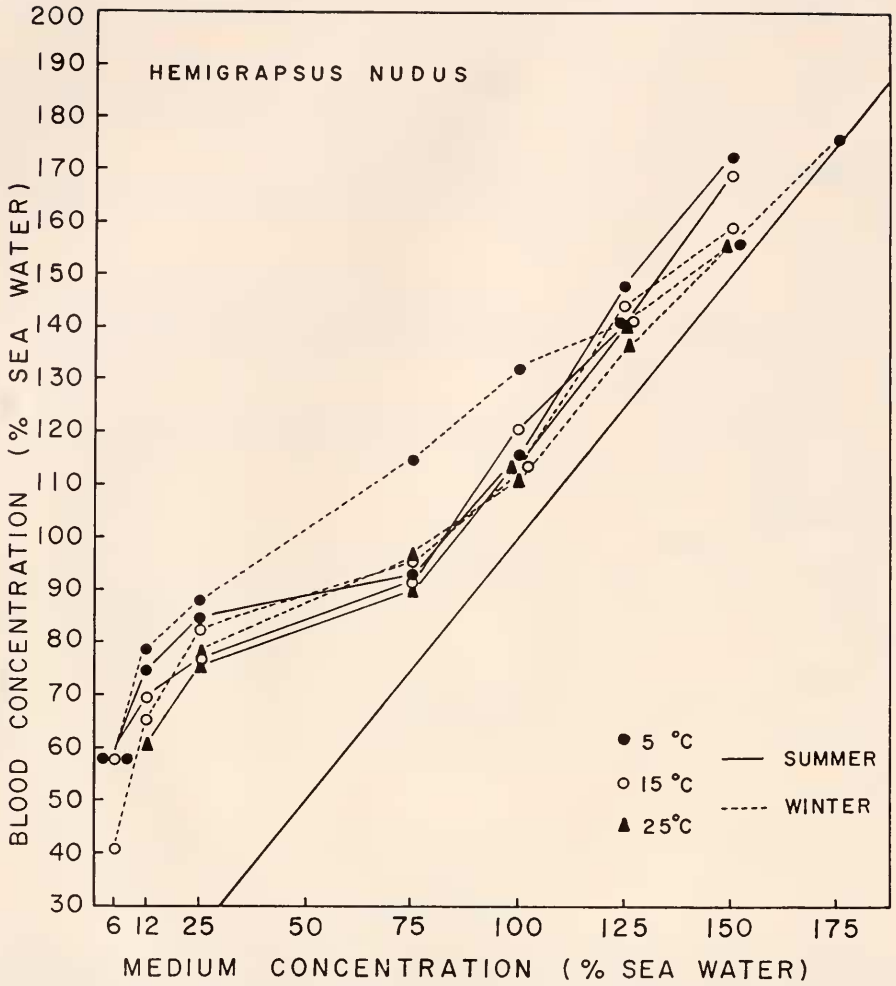

FIGURE 7. Relationship of the body fluid concentration of summer and winter *Hemigrapsus nudus* at 5°, 15° and 25° C., to the medium concentrations after exposure for 48 hours to the experimental salinities.

the intertidal zone, showed the blood concentration always to be higher when compared with summer crabs. And, at either season the blood concentration was higher than the intertidal salinity.

The concentration gradients between blood and external media for summer and winter *H. nudus* are presented in Figure 4. Winter crabs of this species maintained a greater gradient ($P = 0.01$) at sea water concentrations less than 125% except at 6%. At higher concentrations summer animals had the greater gradient ($P = 0.01$). As salinity increased winter crabs steadily decreased their gradient, and attained isotonicity at 175% sea water. The greatest gradient occurred at 12% sea water, for both summer and winter animals. At this low salinity, winter

crabs maintained a 67% gradient and summer crabs a 57% gradient, and this difference is significant. Below 125% sea water, winter crabs are better regulators, and above this concentration, summer animals are better regulators. The differences at each salinity between summer and winter crabs are significant ($P = 0.01$), except at 6% and 125% sea water. Blood concentrations of summer and winter animals are significantly hypertonic ($P = 0.01$) to all external salinities (Fig. 7), except for winter animals at 5° and 25° C., 150% sea water, and 5° C., 175% sea water.

Mortality for summer *H. nudus* was somewhat similar to that reported for *H. oregonensis*. At 175% salinity deaths occurred at all temperatures, following three hours of exposure to the external medium. At 20° and 25° C. the same effect resulted at 150% sea water.

Interspecific comparison

Differences between the two species concern mainly seasonal changes and relative gradients maintained. Blood concentrations of summer *H. oregonensis* at the three temperatures, and salinities below 100% sea water, were similar, but significantly lower than those of winter animals (Fig. 3). Blood of summer *H. nudus* at the three temperatures, and salinities below 100% sea water, also was similar but not significantly lower than that of winter crabs, with the exception of the winter curve at 5° C. (Fig. 7). At salinities higher than 125% sea water, winter blood concentrations of both species were significantly lower and closer to isotonicity than corresponding summer ones.

Data presented in Figure 4 demonstrate clearly specific differences. Winter animals of both species maintained a greater gradient at salinities ranging from 6% to 100%–125% sea water. At higher external sea water concentrations summer animals of both species had the greater gradient. These differences between summer and winter animals of both species compared at each salinity are significant ($P = 0.01$) with the exception of *H. nudus* at 6% and 125% sea water and *H. oregonensis* at 100% sea water. Comparison of summer animals of both species showed that at salinities below 25% sea water *H. nudus* had a slightly greater gradient. From 25% to 75% sea water the two species had the same gradient. Between 75% and 125%, *H. nudus* was greater and at higher salinities, *H. oregonensis* sustained the greater gradient. At two salinities, 100% and 150% sea water, the differences between summer gradients were significantly different. The relationship between winter animals differed from that of summer ones. Between 6% and 50% sea water the gradient for *H. oregonensis* was greater. Over the range, 50% to approximately 150% sea water, the gradient for *H. nudus* was greater, and above 150% the *H. oregonensis* curve rose whereas that one for *H. nudus* decreased to isotonicity. Interspecific winter comparisons at each salinity are significant ($P = 0.01$) except at 150% sea water. Over the major portion of the salinity range, winter *H. nudus* is the better regulator, compared with summer *H. nudus* and summer and winter *H. oregonensis*.

Weight changes

Winter crabs of both species were placed at a series of salinities and at two temperatures (5° and 25° C.) for a 48-hour period, to determine whether an appreciable weight change occurred, particularly at the extremes of the salinity range (6% and 175% sea water). Percentage of body weight (dry and wet weight)

as water was determined, and from these data it was shown that low and high salinities at either temperature had no effect on water gain or loss. Per cent of total body weight as water ranged from 59% to 65%, averaged 62%, and this range was not statistically significant. Animals were weighed within an average error of less than 0.5% of total body weight.

In all of the experimental procedures a weight range was selected to determine whether size affected time to equilibrium at various external sea water concentrations, and whether total osmotic pressure differed in various size groups. There was no evidence to suggest that size had any effect on the response of these two species of crabs to any of the salinity and temperature combinations, winter or summer.

Discussion

Comparative osmoregulatory abilities

The osmotic responses of these two species of crabs are different, and intraspecifically, the osmoregulatory ability changes seasonally. Over the entire salinity range blood concentrations of both species, summer and winter, and at the three experimental temperatures, are hypertonic to these experimental salinities and these differences, for the most part, are significant. As the gradient increases with decreasing salinity (below 100–125% sea water) osmotic regulation is accelerated (Figs. 3 and 7), and from 6% to 75% sea water both species show strong hyper-osmotic regulation.

The gradient between blood and external medium at various sea water concentrations shows a significant seasonal difference (Fig. 4). Gradients maintained by winter *Hemigrapsus oregonensis* and *H. nudus* are much greater, over the salinity range 6% to 100–125% sea water, when compared with those gradients for summer crabs. At higher salinities (100% to 150% sea water) summer crabs maintain a larger gradient. Differences between the two species become apparent when winter gradients are compared. At the lower (6% to 50% sea water) and higher (175% sea water) salinities, winter *H. oregonensis* have a significantly greater gradient. But over the major portion of the salinity range (50% to 150% sea water) winter *H. nudus* maintain the significantly greater gradient. Summer crabs are similar from 6% to 125% sea water. Only at the highest salinity (150% sea water) does *H. oregonensis* sustain a significantly greater gradient than *H. nudus*. The two summer curves are linear and parallel from 25% to 75% sea water; winter curves are nearly linear but not parallel from 12% to 125% sea water. The winter *H. nudus* curves approach linearity over most of the salinity range.

Jones (1941) found that both species of *Hemigrapsus* could regulate in dilute sea water, but even after 72 hours' immersion, he was unable to demonstrate regulation in concentrated sea water. Gross (1957a) confirmed the work of Jones (1941), but demonstrated further that *Hemigrapsus* can hypo-osmoregulate up to 33% (gradient between blood and external medium) for 20 hours in 150% sea water. He had shown that at 75% sea water both species maintain less than a 10% sea water gradient (calculated from Jones, 1941). It is assumed that at this salinity, isotonicity is most closely approached.

The present investigation supports the work of both Jones (1941) and Gross

(1957a), but demonstrates that regulation at higher and lower salinities is dependent upon the season, and blood concentrations are hypertonic to all experimental salinities. Summer *H. oregonensis* maintain a gradient up to 30% at 150% sea water, for longer than a 48-hour period. Summer *H. nudus* maintain one up to 20%. Winter *H. oregonensis* sustain a gradient up to 15% in 175% sea water, for the same period of time, but winter *H. nudus* are isotonic. At 75% sea water, the gradients of both species, summer and winter, particularly the latter, are much higher than those reported by Gross (1957a).

Gross (1960) recently has studied a transient population of *Hemigrapsus oregonensis* in southern California. A lagoon was isolated temporarily from the sea, and over a period of four months the salinity increased to 190% sea water. He has shown that in 168% sea water, this species is capable of hypo-osmotic regulation (gradient was 23%). At higher salinities the blood was nearly isotonic. These data differ from those presented here, in that this northern population is always hypertonic at all salinities. This condition of maintaining blood hypertonic to hypersaline media probably should not be referred to as regulation, in that it deviates from the normal understanding of homeostasis. The fact that per cent body weight as water is not affected by various sea water concentrations suggests that at high salinities, salts are being absorbed from the external medium and this maintains hypertonic blood. This hypertonicity, however, may result from a gradual breakdown of the regulatory mechanism. Gross (unpublished) acclimated a group of *Hemigrapsus oregonensis,* over a period of two months, from a field salinity of 163% sea water to 100% sea water. Following this, these animals were immersed directly into 150% sea water for 72 hours. These animals could not regulate at this salinity. He has suggested that long term acclimation has permitted hypo-osmotic regulation in this species.

Effect of temperature

There is little evidence available regarding the effect of temperature on osmotic regulation. Jones (1941) has shown that a higher temperature causes a slightly higher osmotic pressure. He states (p. 85) that "the magnitude of the change is not great as compared with the individual variations at a given temperature." For instance, over a 20° C. range, the increase in the hyper-osmotic regulation curve for *Hemigrapsus oregonensis* and *H. nudus* is approximately 5% to 7% sea water. Bateman (1933) showed in *Carcinus maenas* an increase in osmotic pressure with an increase in temperature. The increase was slight; with a temperature range of 14° C. the increase was approximately 1% sea water. Widmann (1935) found in *Eriocheir sinensis* an increase in osmotic pressure with a decrease in temperature. But again the increase was small. More recently, Williams (1960) has studied the effect of temperature on osmotic regulation in two shrimps, *Penaeus aztecus* and *P. duorarum.* He reported that as temperature was lowered from 28° to 18° C. and 18° to 8° C., blood concentration approached isotonicity at all experimental salinities (10‰ to 30.5‰). He stated further that at low temperatures (8° C.) the regulatory ability was impaired. It is difficult to compare the response at different temperatures because the time at each temperature was not the same. Gross (unpublished), however, has demonstrated in *Dendrostomum*

that blood Ca and Mg are significantly higher at lower temperatures (3.5° C.) when compared with higher ones (13.5° C.).

From the foregoing data, it would appear that a temperature effect is yet to be clearly documented with the exception of that reported by Gross (unpublished). The seasonal effect on osmotic regulation in *Hemigrapsus* has been demonstrated in this paper (Figs. 3, 4 and 7). These differences are the result of temperature. Within either species, summer animals at lower salinities are consistently lower in blood concentration than winter animals, and at higher salinities, summer animals are consistently higher. Over the salinity range (25% to 75% sea water) encountered seasonally by these populations, the difference between the summer and winter gradient of *H. oregonensis* at 25% sea water is 21%; at 75% sea water, the difference is 10%. The same comparison for *H. nudus,* at 25% sea water, is 8%; at 75% sea water, 23%. The differences in these gradients are significant ($P = 0.01$). The two summer curves from 25% to 75% sea water are identical, and can be used as a baseline (Fig. 4). As salinity increases, low temperature (winter) causes a sharp decrease in the gradient (curve steepens) for *H. oregonensis,* and conversely, low temperature causes a more gradual decrease for *H. nudus.*

Comparison of Figures 3 and 7 shows, in both cases, a difference in the 5° C. winter curve over the salinity range, 25% to 75–100% sea water, depending upon the species, when this curve is compared with all others. Any significance attached to this difference can be determined only after specific ion analyses, research on which is proceeding at present.

Metabolic work in increased osmotic stresses

Recently, studies on whole animal respiration of *Hemigrapsus oregonensis* and *H. nudus,* as influenced by various temperature and salinity combinations, have shown that for a given salinity, animals acclimated to low temperature (5° C.) have a higher rate of metabolism. Further, crabs acclimated to a given temperature have a higher rate at lower salinities (Dehnel, 1960). Weight-specific oxygen consumption is highest at the low acclimation temperature, low acclimation salinity combination. Rates of oxygen consumption remain high as temperature increases in the low salinity for *H. oregonensis,* but high salinity results in a higher rate for *H. nudus.* The greatest gradient maintained by either species, summer or winter, is at a low salinity, 12% sea water (Fig. 4). In order to maintain a gradient of this magnitude (hyper-osmoregulate) some metabolic work must result. This does not define, however, the percentage relative to total metabolism of the whole animal.

Potts (1954) has discussed recently the dynamic aspects of osmoregulation and has pointed out that urine hypotonic to the blood in brackish-water animals has only a slight effect on osmotic work. But, if in fresh water, urine is greatly concentrated relative to the medium, and still hypotonic to the blood, osmotic work is reduced significantly. Both species of *Hemigrapsus* in this geographic area are found only in brackish or estuarine water, and in the summer estuarine conditions become more pronounced (salinity, 25% to 35% sea water). These seasonal changes present ecological conditions intermediate to brackish and fresh water as discussed by Potts (1954). Over the experimental salinity range, blood of winter

animals of both species is always hypertonic to the urine. The blood of summer crabs of both species is isotonic at lower salinities and slightly hypertonic at higher salinities.

It is possible to compare respiration rates and osmoregulatory data at various temperature and salinity combinations in order to determine whether one can accord increased metabolic activity to responses to salinity where large gradients are maintained. Whole-animal respiratory rates for summer animals of both species, at a series of acclimation temperatures and salinities, are available to relate these values to the present data, specifically the hyper-osmotic regulation portion of the salinity curves (Dehnel, 1960). If the osmotic gradients of summer animals, determined at 5° (Figs. 3 and 7) and 20° C. (data not presented) and 25% and 75% sea water, are compared with weight-specific oxygen consumption values for summer animals acclimated to the same temperature and salinity conditions (Dehnel, 1960), the following relations can be shown. For *H. oregonensis* at 5° and 20° C. there is 129% and 157% increase in gradient, respectively, as salinity decreases from 75% to 25% sea water. Metabolic rate accordingly increases as salinity decreases and at either temperature, by essentially a constant value, 28%. The gradient increase for *H. nudus,* compared as above, is 233% at 5° C. and 146% at 20° C. Oxygen consumption at the lower temperature shows a 31% increase, but at 20° C., even though the gradient has more than doubled, respiratory rate has decreased 20%. In all examples the gradient increases as salinity decreases, and except for *H. nudus* at 20° C., there is a parallel increase in oxygen consumption, and the magnitude of increase is approximately the same.

An important reference point is the comparison of blood and urine at various experimental salinities. One aspect of the work currently in progress (to be published elsewhere) concerns changes in total osmotic pressure of the urine of *Hemigrapsus* under identical conditions to those described for blood. When the gradient between the blood and urine is compared seasonally (plotted as in Figure 4), winter *H. oregonensis* and *H. nudus* have blood hypertonic to urine over the entire salinity range. There exists at least a 10% absolute difference, and in some cases the difference is as high as 35%. Blood of summer animals of both species is isotonic with the urine at salinities ranging from 6% to 100% sea water, and is slightly hypertonic at sea water concentrations above 100% sea water. If the gradient between urine and the external medium is compared, winter *H. oregonensis* maintain a greater gradient than summer crabs, at lower salinities, and at higher salinities urine of winter *H. oregonensis* is hypotonic. Above 100% sea water urine of summer crabs is hypertonic to the external medium. The gradient for summer and winter *H. nudus* from 25% to 75% sea water is similar. Above this salinity, summer and winter *H. nudus* follow the same trend as described for summer and winter *H. oregonensis,* although the latter are somewhat higher in urine concentration at each of the respective higher salinities. The general trend for blood and urine is the same with the exception that urine of winter crabs becomes hypotonic at 100% sea water and above, whereas blood concentration always remains hypertonic.

Several considerations arise from comparison of these data. The fact that oxygen consumption increase is approximately the same, whereas the percentage increase in gradient at the low salinity is different, suggests that increased osmotic

work cannot be detected by measuring whole-animal oxygen consumption, at least under these conditions. The exception, namely *H. nudus* at 20° C., documents further the above. Summer gradients of either species at both temperatures compared at 25% or 75% sea water are similar, and interspecifically, these species in the summer hyper-osmoregulate to a comparable degree. This is seen in Figure 4 at 15° C. The reasons for the decrease in oxygen consumption at 25% sea water, 20° C. for *H. nudus* cannot be explained.

Mechanism of osmoregulation

Prosser, Green and Chow (1955) have presented evidence for an osmotic regulatory role of the antennary gland in *Pachygrapsus*. The kidney functions probably only for hyper-osmotic regulation and even more selectively for the regulation of Ca and Mg ions. Green, Harsch, Barr and Prosser (1959) have demonstrated a similar role, especially for Mg ions, for the antennary gland of *Uca*. Gross (1960) has demonstrated high Mg urine concentrations for *H. oregonensis*. The selective role of the kidney for ionic regulation is being determined for these northern populations of the two species of *Hemigrapsus*. These data suggest that the kidney functions for the regulation of Ca and Mg ions, and probably in both low and high salinities.

The role of the gill for osmotic regulation is well documented. Gross (1957a) has demonstrated a flux of salts and water in the gill chamber of *Pachygrapsus*. Green, Harsch, Barr and Prosser (1959) have shown that water and ions enter via the gills and stomach of *Uca* and the sites of regulation are the gills and the antennary gland (see above). There are no data available at present to suggest the role of the gill in osmotic regulation in *Hemigrapsus*. In this laboratory, rate of respiration of gill tissue has been determined for summer and winter crabs of both species, over a range of temperatures and salinities. These data show that gill tissue respiration of crabs measured at a series of acute temperatures, and acclimated to 5°, 12.5° and 20° C. and 35% to 125% sea water, decreased generally as salinity increased, and the decrease was greatest in summer animals acclimated to 5° C.

There were no detectable weight changes in winter crabs in the experiments conducted at two temperatures (5° and 25° C.) and at eight salinities (6% to 175% sea water). Per cent of the total body weight as water was approximately 62%. These data indicate that there was no net gain or loss of water, but if water does change blood concentration, its effect is negligible. This is an agreement with Hukuda (1932) and Gross (1957a). If, then, water fluxes do not occur, it is reasonable to assume that body fluid concentration changes result from salt movement. At the lower salinities (6% to 75% sea water) salt concentration of the blood was maintained actively against a gradient in both species, winter and summer (Figs. 3 and 7). Between 75% and 100% sea water, the blood continued to maintain a gradient, although this gradient decreased as the salinity increased. Blood of animals removed directly from field conditions was essentially 100% sea water, even though field salinities, summer and winter, are approximately 25% and 75% sea water, respectively. At experimental salinities above 100% sea water, blood concentrations remained hypertonic and were maintained against a gradient.

The present data permit no further interpretation of the above results. Work

currently in progress on major ion changes in blood and urine for the two species and at the various temperature and salinity combinations will demonstrate the selective role of the kidney, temperature effect on ion exchange, and the hypertonic condition of the blood at higher salinities.

Intertidal distribution

It has been suggested that seasonal and laboratory responses of *Hemigrapsus nudus* to combinations of temperature and salinity, as measured by whole animal oxygen consumption and temperature tolerance, might explain the establishment of this species in this geographic area (Dehnel, 1960). Winter *H. nudus* maintain a greater gradient when compared with *H. oregonensis,* over an approximate salinity range, 50% to 150% sea water. At lower and higher salinities, *H. oregonensis* has a higher gradient. Gradients for summer populations of both species were approximately the same (Fig. 4). Based on these gradients *H. nudus* is the better regulator.

Gross (1957b) has demonstrated that *Pachygrapsus crassipes* prefers 100% sea water to other concentrations, ranging from 50% to 150% sea water. This preference was not altered by desiccation or acclimation to low salinity, but could be changed with high salinity acclimation. He suggests that this preference may serve as a mechanism for limiting *Pachygrapsus* to the intertidal zone.

Studies on salinity preference have shown that winter *H. nudus* has no preference for the three salinities tested, 25%, 75% and 125% sea water, whereas *H. oregonensis* showed a distinct preference for 75% sea water, the field salinity for winter animals.

Gross (1957a) has found a direct correlation between exoskeleton permeability and osmotic regulation in a series of crabs, habitats of which range from marine to terrestrial. In this series of crabs, the exoskeleton of *H. nudus* was shown to be less permeable than that of *H. oregonensis.*

Winter breeding of both species of *Hemigrapsus* occurs at this latitude. Zoea and megalops larvae of *H. nudus* from the open coast could have introduced this species into this geographic area, and winter temperature and salinity conditions would not restrict the establishment of a population in the intertidal region. The better regulatory ability of *H. nudus,* lack of salinity preference for winter animals, in conjunction with the less permeable exoskeleton, would allow this species to locate higher in the intertidal regions, where there is a greater osmotic stress, but reduced competition with the population of *H. oregonensis* that is established in the lower tidal areas. Habitat requirements of the two species differ slightly, but are not sufficient to restrict considerable overlap of the two populations.

Summary

1. Total osmotic pressure measurements of blood were made on two species of intertidal crabs, *Hemigrapsus oregonensis* and *H. nudus,* over a salinity range, 6% to 175% sea water, a temperature range, 5° to 25° C., and at two seasons, summer and winter.

2. Major changes in blood concentration occurred at 48 hours. Both species at either season were hypertonic to all experimental salinities. Below 100% sea

water, osmotic regulation was accelerated with decreasing salinity, and in hypotonic media both species showed strong hyper-osmotic regulation.

3. The osmoregulatory abilities of these two species changed seasonally, and these responses resulted from the effect of temperature. Winter crabs maintained greater gradients at sea water concentrations below 100–125% sea water, and were better regulators in hypotonic media, whereas summer crabs were better regulators in hypertonic media, and consequently maintained the greater gradient. Interspecifically, winter *H. nudus* maintained the greater gradient over the major portion of the salinity range. Summer crabs of both species were similar from 6% to 125% sea water.

4. Blood concentrations of *H. oregonensis,* when measured at a series of temperatures and at each salinity, showed a general trend, particularly for winter animals. As external sea water concentration decreased (from 75% to 12% sea water) blood concentrations increased significantly with decreasing temperature. Blood concentrations of summer animals measured at the three temperatures and each salinity showed no real difference, but at the lower salinities, blood concentrations of summer animals were significantly lower than those of winter crabs. The same general trend was shown for *H. nudus.* Temperature had no effect on blood concentrations of summer animals, but the low temperature (5° C.) had a highly significant effect on the blood concentrations of winter animals.

5. No detectable weight changes resulted when animals were subjected to the extreme experimental sea water concentrations. Further, there was no evidence to suggest that size had any effect on osmotic response.

6. Respiratory and osmoregulatory data for both species have been compared at the same temperatures and salinities to determine whether increased osmotic work can be resolved by measuring oxygen consumption. Comparison of these data does not permit such a conclusion.

LITERATURE CITED

Bateman, J. B., 1933. Osmotic and ionic regulation in the shore crab, *Carcinus maenas,* with notes on the blood concentrations of *Gammarus locusta* and *Ligia oceanica. J. Exp. Biol.,* **10**: 355–371.

Dehnel, P. A., 1960. Effect of temperature and salinity on the oxygen consumption of two intertidal crabs. *Biol. Bull.,* **118**: 215–249.

Flemister, L. J., and S. C. Flemister, 1951. Chloride ion regulation and oxygen consumption in the crab *Ocypode albicans* (Bosq). *Biol. Bull.,* **101**: 259–273.

Green, J. W., M. Harsch, L. Barr and C. L. Prosser, 1959. The regulation of water and salt by the fiddler crabs, *Uca pugnax* and *Uca pugilator. Biol. Bull.,* **116**: 76–87.

Gross, W. J., 1954. Osmotic responses in the sipunculid *Dendrostomum zostericolum. J. Exp. Biol.,* **31**: 402–423.

Gross, W. J., 1955. Aspects of osmotic regulation in crabs showing the terrestrial habit. *Amer. Nat.,* **89**: 205–222.

Gross, W. J., 1957a. An analysis of response to osmotic stress in selected decapod Crustacea. *Biol. Bull.,* **112**: 43–62.

Gross, W. J., 1957b. A behavioral mechanism for osmotic regulation in a semi-terrestrial crab. Biol. Bull., **113**: 268–274.

Gross, W. J., 1958. Potassium and sodium regulation in an intertidal crab. *Biol. Bull.,* **114**: 334–347.

Gross, W. J., 1959. The effect of osmotic stress on the ionic exchange of a shore crab. *Biol. Bull.,* **116**: 248–257.

Gross, W. J., 1960. Hypo-osmotic regulation in two species of crab. *Anat. Rec.,* **137**: 360–361.

Hukuda, K., 1932. Change in weight of marine animals in diluted media. *J. Exp. Biol.*, **9**: 61–68.

Jones, L. L., 1941. Osmotic regulation in several crabs of the Pacific Coast of North America. *J. Cell. Comp. Physiol.*, **18**: 79–92.

Krogh, A., 1939. Osmotic Regulation in Aquatic Animals. Cambridge at the University Press.

Potts, W. T. W., 1954. The energetics of osmotic regulation in brackish and freshwater animals. *J. Exp. Biol.*, **31**: 618–630.

Prosser, C. L., and F. A. Brown, Jr., 1961. Comparative Animal Physiology. W. B. Saunders Co., Philadelphia.

Prosser, C. L., J. W. Green, and T. J. Chow, 1955. Ionic and osmotic concentrations in blood and urine of *Pachygrapsus crassipes* acclimated to different salinities. *Biol. Bull.*, **109**: 99–107.

Schlieper, C., 1929. Ueber die Einwirkung nieder Salzkonzentrationen auf marine Organismen. *Zeitschr. vergl. Physiol.*, **9**: 478–514.

Schwabe, E., 1933. Ueber die Osmoregulation verschiedener Krebse (malacostracen). *Zeitschr. vergl. Physiol.*, **19**: 183–236.

Widmann, E., 1935. Osmoregulation bei einheimischen Wasser- und Feuchtluft-Crustaceen. *Zeitschr. wiss. Zool.*, **147**: 132–169.

Wikgren, Bo-Jungar, 1953. Osmotic regulation in some aquatic animals with special reference to the influence of temperature. *Acta Zool. Fennica*, **71**: 1–102.

Williams, A. B., 1960. The influence of temperature on osmotic regulation in two species of estuarine shrimps (*Penaeus*). *Biol. Bull.*, **119**: 560–571.